ASSESSING THE EFFECTS ON BLOOD PRESSURE OF VIETNAM GREEN COFFEE BEAN EXTRACT ON EXPERIMENTAL ANIMALS

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ABSTRACT: *This is a prospective descriptive cross-sectional study of the effects of green coffee bean extract on types of blood pressure components of experimental mice before and after strenuous exercise on the treadmill.*

Results: *Systolic blood pressure, diastolic blood pressure, pulse pressure and mean blood pressure before and after strenuous exercise in the group of mice drinking green coffee bean extract at a dose of 400 mg/kg and a part of the group of mice drinking it at a dose of 300 mg/kg increased less than the group of mice drinking physiological saline.*

This result initially showed that green coffee bean extract at doses of 300 mg/kg and 400 mg/kg had protective effects on cardiovascular function and against fatigue in experimental animal models.

Keywords: Green coffee bean extract, blood pressure, mice.

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1. INTRODUCTION.

Green coffee bean extract has been shown to have many beneficial effects on human health. The beneficial effects have been proven by authors around the world, including: anti-dyslipidemia effect [1, 2], weight loss, TNF-α reduction [2], Glycemia reduction [3]… In particular, the effect of reducing blood pressure during strenuous exercise has also been demonstrated in healthy people [4]. These effects may be related to the chemical constituents of green coffee bean extract. However, the ratio of these components is closely related to the soil and climate where coffee is grown [5].

In Vietnam, coffee is a fairly popular drink in Vietnam, especially in the South and other urban areas. Coffee is grown quite commonly in the Central Highlands and South Central of our country. This will be an important source of raw materials, with a large reserve for extracting and processing into green coffee bean extract to treat a number of human diseases.

As a basis for clinical application, we conducted this study to evaluate the effects of green coffee bean extract on various types of blood pressure components in experimental rats before and after performing the running test on the treadmill.

2. SUBJECTS AND METHODS OF THE STUDY.

2.1. Research subjects and materials:

- Research subjects: 40 Swiss white mice, weight ranging from 20 to 24 grams. Mice were raised in cool conditions with a 12-hour light-dark cycle and no restrictions on food and water. All animal care and use procedures strictly follow the guidelines for animal care and use of the Military Medical Academy.

- Research materials: Samples of Moka green coffee beans (*C. arabica var. mokka*) were harvested from gardeners in Da Lat, Lam Dong in December 2016. Fresh coffee berries are peeled after being brought back, and the seeds are dried (for about 5 days). Then, grind the seeds into a powder, store them at room temperature in a cool place. Green coffee bean powder was extracted by ultrasonic extraction and Soxhlet extraction; enriched with liquid-liquid extraction through hexane, chloroform and EtOAc solvents to form the extract.

2.2. Methods:

- Study design: prospective, experimental, cross-sectional description.

- 40 mice were randomly divided into 4 groups, 10 animals for each group:

+ Group 1 (control group): mice were treated with physiological saline.

+ Group 2 (treatment group 1): mice were given green coffee bean extract, a dose of 200 mg/kg.

+ Group 3 (treatment group 2): mice were given green coffee bean extract, a dose of 300 mg/kg.

+ Group 4 (treatment group 3): mice were given green coffee bean extract, a dose of 400 mg/kg.

Rats were given the study substance and physiological saline 5 times a week, continuously for 3 weeks before performing strenuous exercises.

- Inducing exercise model on mice: the experiment was conducted on a treadmill with many running belts with a width of 6cm, a length of 20cm with adjustable speed (Figure 1). The research groups were put on a running belt and had to run continuously for 15 minutes at a speed of about 30 rpm.

Figure 1. Treadmill for mice.

- Evaluating mouse tail's blood pressure: mouse blood pressure is measured through a manometer connected to the Powerlab data logging system (ADInstrument, Australia - Figure 2). The mouse is fixed in a plastic holder. The tail of the mouse is attached to the blood pressure sensor of the Powerlab system for indirect blood pressure recording. Mouse tail's blood pressure was recorded at the time points before and after exercise 1 min, 3 min, and 5 min. Blood pressure was analyzed offline using Labchart 8.0 software.

+ The pulse pressure was calculated using the formula:

Pulse Pressure = Systolic Pressure - Diastolic Pressure.

+ Mean Blood Pressure:

Mean Blood Pressure = Diastolic Pressure + (Systolic Pressure - Diastolic Pressure)/3

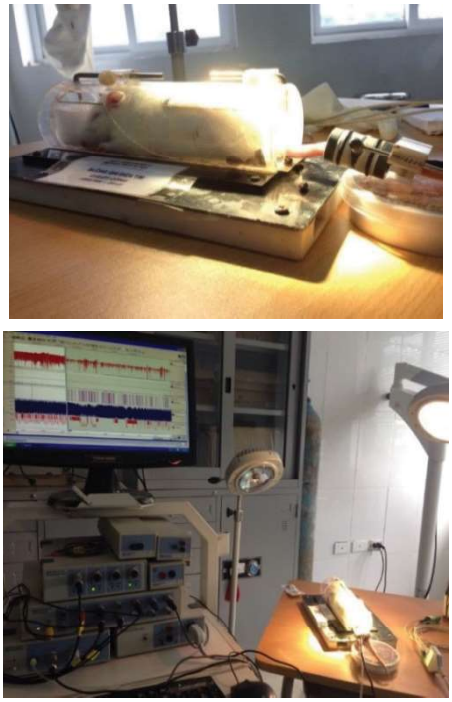

Figure 2. Mouse tail's blood pressure recording using the ADInstrument system

- Data analysis: Data were analyzed using SPSS 20.0 software. A statistically significant difference was determined with $p<0.05$.

3. RESULTS.

3.1. A blood pressure sample was recorded from a mouse:

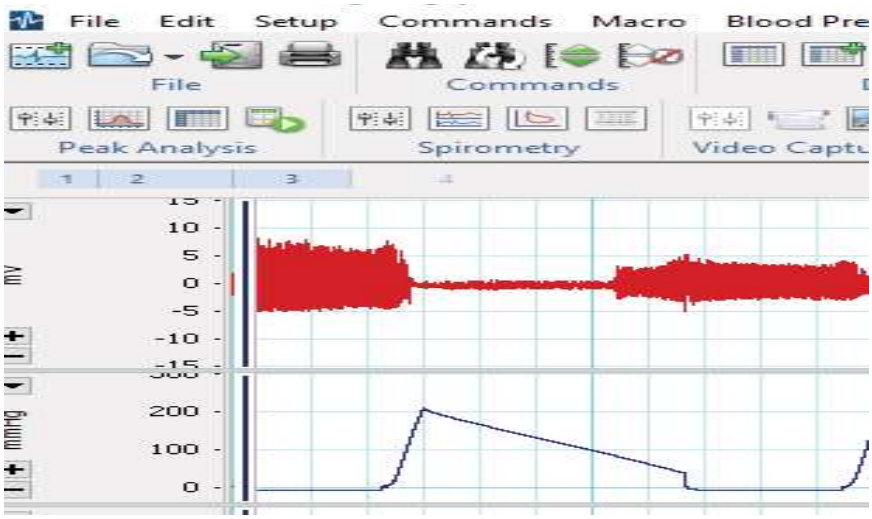

Figure 3. A blood pressure sample was recorded from a mouse.

3.2. Systolic pressure:

Table 1. Systolic pressure before and after the running test (mmHg).

Groups	Before and after the running test				p
	Before[a]	After 1 min[b]	After 3 min[c]	After 5 min[d]	
Control[1]	113.30 ± 11.88	134.81± 10.12	138.55 ± 17.74	144.94 ± 15.40	$p_{b,c,d-a} < 0.001$
200 mg/kg[2]	113.44 ± 13.95	132.28 ± 12.67	136.74 ± 16.05	143.22 ± 15.15	$p_{b,c,d-a} < 0.01$
300 mg/kg[3]	113.09 ± 11.68	130.67 ± 14.00	134.29 ± 15.71	139.44 ± 12.63	$p_{b,c,d-a} < 0.01$
400 mg/kg[4]	112.95 ± 11.53	127.60 ± 13.42	128.92 ± 13.98	131.35 ± 9.76	$p_{b,c,d-a} < 0.05$
p	p > 0.05	p > 0.05	p > 0.05	$p_{3-1} < 0.1$ $p_{4-1} < 0.05$	

Systolic blood pressure increased after exercise with a statistically significant difference between before and after exercise ($p < 0.05$, $p < 0.01$, and $p < 0.001$). Comparison between study groups showed that before exercise and after exercise 1 minute and 3 minutes, there was no statistically significant difference in systolic blood pressure between the study groups ($p > 0.05$). At the time of exertion of 5 minutes, systolic blood pressure in the group drinking 400 mg/kg dose was statistically significantly lower than the control group ($p < 0.05$) and in the group taking the green coffee extract. Green coffee beans at a dose of 300 mg/kg were significantly lower than the control group ($p < 0.1$).

3.3. Diastolic pressure:

Table 2. Diastolic pressure before and after the running test (mmHg).

Groups	Before and after the running test				p
	Before[a]	After 1 min[b]	After 3 min[c]	After 5 min[d]	
Control[1]	81.52 ± 6.00	91.92 ± 3.97	93.68 ± 2.96	93.58 ± 4.30	$p_{b,c,d-a} < 0.001$
200 mg/kg[2]	81.57 ± 6.09	89.83 ± 2.78	92.22 ± 3.41	94.50 ± 3.46	$p_{b,c,d-a} < 0.01$
300 mg/kg[3]	81.21 ± 7.87	87.83 ± 6.59	89.95 ± 3.99	93.05 ± 4.02	$p_{b,c,d-a} < 0.01$
400 mg/kg[4]	81.01 ± 7.60	85.25 ± 4.33	84.95 ± 7.46	85.74 ± 4.95	$p_{b,c,d-a} < 0.05$
p	p > 0.05	$p_{4-1} < 0.01$	$p_{4-1,2,3} < 0.05$	$p_{4-1,2,3} < 0.05$	

Diastolic blood pressure increased after exercise with a statistically significant difference between before and after exercise ($p < 0.05$, $p < 0.01$, and $p < 0.001$). Comparing between study groups, our study results show that before exercise, there is no statistically significant difference in diastolic blood pressure between the study groups. However, after exertion, the diastolic blood pressure in the group drinking green coffee bean extract 400 mg/kg dose was statistically significantly lower than the control group, the group drinking green coffee bean extract 200 mg/kg dose and 300 mg /kg dose ($p < 0.05$ and $p < 0.01$).

3.4. Pulse pressure:

Table 3. Pulse pressure before and after the running test (mmHg).

Groups	Before and after the running test				p
	Before[a]	After 1 min[b]	After 3 min[c]	After 5 min[d]	
Control[1]	31.77 ± 12.92	42.89 ± 11.81	44.87 ± 17.75	51.36 ± 14.48	$p_{c,d-a} < 0.05$
200 mg/kg[2]	31.87 ± 11.49	42.44 ± 13.75	44.51 ± 15.29	48.71 ± 15.27	$p_{c,d-a} < 0.05$
300 mg/kg[3]	31.88 ± 11.42	42.83 ± 13.37	44.34 ± 14.76	46.39 ± 11.80	$p_{c,d-a} < 0.05$
400 mg/kg[4]	31.94 ± 10.11	42.35 ± 11.57	43.97 ± 11.33	45.62 ± 7.54	$p_{c,d-a} < 0.05$
p	p > 0.05	p > 0.05	p > 0.05	p > 0.05	

The pulse pressure after exertion 3 minutes and 5 minutes was statistically significant compared with before exercise ($p < 0.05$). Comparing between the study groups, our study results show that although the diastolic blood pressure in the group drinking green coffee bean extract increased less than the control group, there was no statistically significant difference in diastolic blood pressure between the study groups before and after exercise ($p > 0.05$).

3.5. Mean blood pressure:

Table 4. Mean blood pressure before and after the running test (mmHg).

Groups	Before and after the running test				p
	Before[a]	After 1 min[b]	After 3 min[c]	After 5 min[d]	
Control[1]	92.12 ± 5.82	106.22 ± 3.70	108.64 ± 6.38	110.70 ± 6.69	$p_{b,c,d-a} < 0.001$
200 mg/kg[2]	92.19 ± 7.76	103.98 ± 4.08	107.06 ± 6.45	110.74 ± 5.72	$p_{b,c,d-a} < 0.001$
300 mg/kg[3]	91.84 ± 7.60	102.11 ± 7.39	104.73 ± 6.67	108.51 ± 5.74	$p_{b,c,d-a} < 0.001$
400 mg/kg[4]	91.65 ± 7.75	99.36 ± 6.54	99.61 ± 8.59	100.94 ± 5.95	$p_{b,c,d-a} < 0.01$
p	p > 0.05	$p_{4-1} < 0.05$	$p_{4-1} < 0.01$	$p_{4-1,2,3} < 0.05$	

Mean blood pressure increased after exercise with a statistically significant difference compared to before exercise in all study groups ($p < 0.05$ and $p < 0.01$). Comparing with the study groups, the results showed that the mean blood pressure in the green coffee bean extract group increased less after exercise compared with the control group. In which, before exercise, there was no statistically significant difference in mean blood pressure between the study groups ($p > 0.05$). After trying for 1 minute and 3 minutes, the mean blood pressure in the group drinking green coffee bean extract at a dose of 400 mg/kg was statistically significantly lower than in the control group ($p < 0.05$). At the time of 5 minutes after exercise, the mean blood pressure in the group taking the 400 mg/kg dose of coffee bean extract was statistically significantly lower than the control group, the 200 mg/kg green coffee bean extract group and 30 mg/kg ($p < 0.05$).

4. DISCUSSION.

In this study, we evaluated the change in blood pressure after exercise under the influence of green coffee bean extract in experimental rats. There are 4 different types of blood pressure components, including systolic blood pressure, diastolic blood pressure, difference blood pressure and mean blood pressure [1, 5]. Each of these blood pressure values has different clinical significance and value.

- Systolic blood pressure: all groups of rats have increased systolic blood pressure after exercise, but in the control group, the increase in systolic blood pressure is the most. In particular, the increase in systolic blood pressure in the group of rats drinking green coffee bean extract at a dose of 400 mg/kg compared with the control group after 5 minutes of exercise was significantly lower than that of the control group. This result is similar to that observed in some previous studies (systolic blood pressure in the green coffee group increased less than in the black coffee group [8] or placebo [9]). Systolic blood pressure represents the force of contraction of the heart muscle. When systolic blood pressure rises too high, the heart will work under conditions of great stress, which can quickly affect heart function. This strenuous activity will not be able to last for a long time, easily causing a feeling of fatigue after a period of exertion. So, when systolic blood pressure rises less, there is less fatigue in the body, which can prolong periods of high cardiac activity. From there, increase the exercise capacity in experimental animals as well as in humans.

- Diastolic blood pressure: the results of our study showed that green coffee bean extract made diastolic blood pressure increase less in rats after exercise, especially in the group of mice that drank coffee green bean extract at a dose of 400mg/kg. Diastolic blood pressure represents peripheral resistance [6]. Therefore, an increase in diastolic blood pressure will decrease the ejection fraction, and decrease the systolic volume. Thereby, reducing the efficiency of the heart's contraction, reducing the capacity of exertion and labor, and easily causing fatigue. Normally, during exercise, diastolic blood pressure usually changes a little [7]. The results of our study also showed that diastolic blood pressure increased little after exercise, especially after 1 minute of exercise. The diastolic blood pressure in the group of rats that drank green coffee bean extract at a dose of 400 mg/kg was statistically significantly lower than that of the control group after exercise. This result shows that this high dose of green coffee bean extract has reduced peripheral resistance, increased ejection fraction, made the heart's exercise capacity better, and has a role in combating fatigue during exertion, especially in prolonged exertion. This was further confirmed when we analyzed the difference in blood pressure of the rats before and after exercise.

- Pulse Pressure: pulse pressure increased after exercise in all groups of rats studied, but the difference was not statistically significant between groups. Pulse pressure is the mean

of the systolic blood pressure and the diastolic blood pressure. Normally, the pulse pressure is in the range of 30-50 mmHg. If it falls outside of this range, it is considered either trapped blood pressure (pulse pressure < 30 mmHg) or dilated (pulse pressure > 50 pulse pressure mmHg), which makes the heart less efficient [6]. Here, after 5 minutes of physical exertion in the control group, a dilated blood pressure appeared - not beneficial for the heart's performance. However, pulse pressure was statistically significant in all 4 study groups before exercise and after exercise for 3 minutes, 5 minutes. Therefore, the increase in systolic and diastolic blood pressure in the study groups did not significantly affect the ejection fraction between systolic and diastolic blood pressure. Rats given green coffee bean extract had less increase in systolic and diastolic blood pressure but still ensured the ejection fraction under stress conditions, so it would help the heart avoid fatigue during prolonged exertion, increase the exercise capacity of the heart and body.

- With mean blood pressure: according to the researchers, mean blood pressure is the fictitious blood pressure, which exists stably between systole and diastole in the contractile activity of the heart. This is considered effective blood pressure. When the average blood pressure is low, the vital organs in the body do not receive enough blood, causing the function of these organs to decline rapidly [8]. However, if the average blood pressure is too high, it will not be beneficial for the body. It has been shown that high mean blood pressure, especially above 100 mmHg in the mother during the first 3 months of pregnancy, increases the risk of preeclampsia [9]. In this study, we found that green coffee bean extract resulted in a mean increase in blood pressure less than after exercise. Therefore, it can be said that green coffee bean extract at a dose of 400mg/kg is beneficial for cardiovascular activity and organ function in the body under stress conditions.

5. CONCLUSIONS.

A prospective, cross-sectional study on the effects of Vietnamese green coffee bean extract on different types of blood pressure in experimental animals before and after strenuous activity. Results: systolic blood pressure, diastolic blood pressure, blood pressure difference and mean blood pressure after strenuous exercise in the group of mice drinking green coffee bean extract at a dose of 400 mg/kg and part of the group of mice drinking 300 mg/kg dose increased less than the group of rats drinking physiological saline.

This result initially shows that green coffee bean extract at a dose of 300 mg/kg and a dose of 400 mg/kg has the effect of stabilizing blood pressure and protecting cardiovascular function and anti-fatigue in experimental animal models.

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